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Highlights

Puzzling mRNA: Alternative splicing fine-tunes specificity and function

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ABSTRACT

In this issue of the *Biomedical Journal* we discover how alternative splicing might modulate the cell-type specificity and exact function of a ubiquitous purinergic receptor and how the beta blocker propanolol can contribute to breast cancer therapy. Moreover, we learn which culture conditions generate the best vascularisation of tissue engineered bone and which are the clinical features of acute necrotising encephalopathy in adults. Other studies reveal, how laser irradiation can fix fractured all-ceramic dental restorations *in situ*, and finally that nuclear magnetic resonance holds great potential for the rapid detection of pathogens.

Spotlight on review

Puzzling mRNA: alternative splicing fine-tunes specificity and function

Alternative splicing refers to the process of variable assembly of one gene's exons giving rise to a multitude of messenger RNA (mRNA) isoforms and thus different peptides or non-coding RNAs. This mechanism, present in all eukaryotes, manifolds the diversity of possible gene products, increases information storage efficiency, contributes to evolutionary flexibility and allows for functional fine-tuning in different cell types or developmental stages of the same organism.

Known since 1977 and honoured with a Nobel Prize in 1993, the field received a significant boost from the democratisation of high-throughput sequencing techniques over the past two decades, allowing for assessing the full extent of transcript diversity and their correlation with different cell types, states and diseases [1]. These have notably shown that RNA mis-splicing underlies a broad number of pathologies [2].

Benzaquen et al. choose the example of the purinergic P2RX7 receptor to illustrate how alternative splicing could theoretically influence a large panel of features, such as sub-cellular localisation, homo- *versus* heterodimerisation, function and functionality [3].

P2RX7 recognises extracellular ATP (eATP), and like all types of nucleotides or nucleosides outside of cells, this is rather bad news, as it notifies cells of nearby death and danger due to injury or infection [4].

The main addressees are immune, epithelial and endothelial cells [5], yet all mammalian cells express P2RX7 mRNA, but only a subset displays the functional protein in the cell

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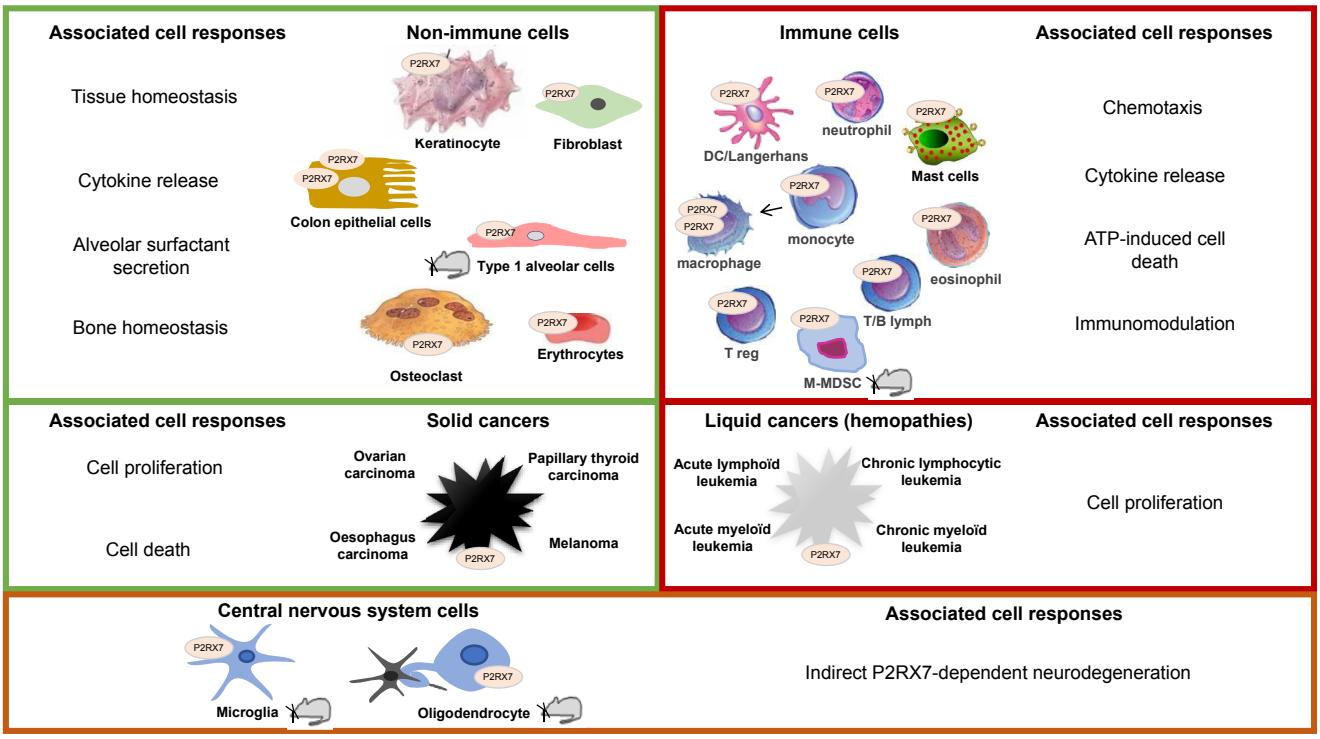

Fig. 1 **The diversity of P2RX7 receptor expression and function.** P2RX7 expression has been documented in a wide range of eukaryotic cells, both immune and non-immune. Their localisation, level of activity and downstream effects differ however among cell types. Cancer cells might overexpress P2RX7 in order to promote proliferation. Source: Benzaquen et al. [3].

membrane. The downstream effects of P2RXT activation are broad, including cell death, proliferation or immune system activities [Fig. 1].

To start with, the authors recapitulate in a comprehensive manner the complex molecular ballet of splicing, from the description of the molecular participants of the "spliceosome" to the many possibilities to combine different exons, introns, promoters and polyadenylation sites into an impressive diversity of mRNAs.

Subsequently, they discuss the intriguing structural features of the P2RX7 protein. The presence of eATP triggers trimeric receptors to form a channel letting mainly potassium exit and calcium enter the cell, inducing membrane depolarisation. However, the role of many features, such as the long C-terminal tail or intra-cytoplasmic sequences, is yet unclear.

Following this, Benzaquen et al. hypothesise how the many splice variants arising from the 13 exons of the human and mouse P2RX7 gene could tune the amplitude of the eATP-induced cell response. For example, a heterotrimer made of the A and B variants seems to have a better plasma membrane expression and ion-channel activity compared to the A version alone, while the association of the A and J forms has a dominant negative effect over the A-homotrimer. It is thus not unlikely that the receptor concentration at the plasma membrane, the sensitivity to eATP and the downstream response are tuned by the respective proportion of different splice variants. Solid experimental proof still needs to be provided however, according to the authors.

Moreover, a similar logic would explain how a P2RX7 version unable to trigger cell death but promote proliferation would be beneficial for cancer cells and fit the observed overexpression of the receptor in several cancers.

Consequently, the authors conclude their review with a connection to the thriving field of onco-immunology by pointing out that tumour-specific splicing products signify the presence of tumour-specific antigens, which could be targeted either by a boosted host immune system or laboratory-made antibodies.

Spotlight on original articles

Promising properties of propanolol in breast cancer treatment

The use of "beta blockers", antagonists of beta adrenergic receptors (β-ARs), is commonly associated with the control of hypertension or abnormal heart rhythms [6]. These G-protein coupled receptors are bound by adrenaline and noradrenaline, and mainly stimulate the sympathetic nervous system [7]. However, it turns out that they are also over-expressed by breast cancer cells [8], which unexpectedly granted beta blockers a complete new field of potential applications [8]. Propanolol, sold for example as Inderal, is one such medication and has been in use since 1964 [9]. Nowadays, it is regarded as one of the safest and most effective medicines according to the World Health Organisation.

In a previous publication based on a retrospective analysis, Alexa Montoya et al. had already shown that propanolol treatment slows down tumour proliferation in early stage

breast cancer patients [10]. In the present publication, the group assesses the impact of propanolol treatment on cell proliferation and apoptosis, in parallel on a late stage breast cancer patient and the MDA-MB-231 breast cancer cell line [11].

Data on the patient's response to the treatment were obtained by comparing the pre-treatment diagnostic biopsy with tumour tissue from a mastectomy after 25 days of propanolol treatment. In addition, as to mimic physiological conditions, the *in vitro* cell culture experiments were carried out with sub-lethal but physiologically attainable plasma concentrations of propanolol.

The authors observe a clear reduction in cancer cell proliferation both in the tumour and the cell culture after propanolol treatment evaluated by Ki-67 staining on the tissues and flow cytometry cell cycle analysis on the cell line, corroborated by a reduction in cyclin expression. Furthermore, a significant effect on cell survival is noted, evident by an increase in p53 protein levels *in vivo* and *in vitro*, as well as the phosphorylation of p53 residues leading to the stabilisation of the latter. In concordance with this observation, levels of the pro-survival protein Bcl-2 are decreased in the treated tissue and cells, strongly hinting that propanolol induces apoptosis in the cancer cells. An increase of the caspase 3, 6 and 9 cleavage products and the sub-G1 cell cycle population as well as the presence of phosphatidylserine on the outer cell surface after treatment of the cell line confirms this diagnostic.

Propanolol has the major advantage that it is a well-tested, non-toxic and inexpensive medication. Having demonstrated its anti-proliferative and pro-apoptotic effect on both early and late breast cancer, Montoya et al. plead strongly for a systematic use of the drug in the framework of breast cancer treatment in order to potentiate other therapies and for an investigation if it could also display similar effects on other cancer types [12].

Also in this issue

Original articles

Optimisation of pre-vascularisation for bone tissue engineering
Although bone tissue possesses remarkable inherent repair and regeneration abilities, the latter can fail after certain complicated fractures, or because of innate defects. As a consequence, bone is the second most frequently transplanted tissue after blood. Grafting materials traditionally include bone auto-, allo- or xenografts, or graft substitutes, each with their advantages and drawbacks. Autografts have the best osteogenic properties but are limited by donor site morbidity and availability, allografts bear the risk of rejection, and xenografts carry the danger of transmission of zoonotic diseases. In this context, *in vitro* tissue engineering has developed as a promising new option for tailor-made bone grafts over the last decade, with cutting edge tools such as stem cells and 3D tissue printing [13]. However, the generation of large grafts poses new problems, for example poor cell viability in the tissue core due to lacking perfusion. In order to circumvent this, pre-vascularisation is achieved *via* the co-culture of endothelial cells with the mesenchymal stem cells (MSCs) meant for differentiation into bone.

Here, Deegan et al. compare different aggregation techniques and culture conditions of MSCs mixed with 5% human umbilical vein endothelial cells (HUVECs) in order to determine their effect on endothelial cell arrangements [14].

The authors find that the best results for endothelial cell regional arrangements are obtained by suspension culture aggregates cultured under hydrostatic loading. Moreover, they show that under these conditions, a low percentage of HUVECs is sufficient for them to be stimulated into tissue-like vascular arrangements.

Characteristics of adult necrotising encephalopathy in adults
Acute necrotizing encephalopathy (ANE) is a rare, fulminant type of encephalopathy, typically preceded by a viral infection. It initially manifests as multifocal, symmetric, oedematous brain lesions mainly in the thalami, brain stem, cerebral white matter, and cerebellum, with the oedema gradually resolving into haemorrhage and necrosis [15]. Given the rarity of the condition, little is known about any typical inflammation marker profile associated with ANE. Furthermore, as the disease is the most common in children under age 5, it is still unclear if adult ANE cases display the same clinical features as in paediatric patients. In order to contribute to resolving this open question, Lin et al. investigate the data of several adult ANE patients [16]. Their study reveals that adult ANE patients indeed manifest clinical symptoms, laboratory data, and neuroimaging findings similar to paediatric cases. However, adult ANE patients are biased towards females and have higher mortality and neurological sequelae rates. In addition, the analysis points towards a role of IL-6 and VCAM-1 in adult ANE pathogenesis, and a potential role of TGF-β for treatment.

Nuclear magnetic resonance proves its worth in specific pathogen detection
Fast, cheap, and specific detection of microbial pathogens in food is of major importance for both the agroalimentary industry and health services. However, FDA-enforced methods tend to be culture- or PCR-based, thus time-consuming or expensive. Seafood, which is often consumed raw or undercooked, is a frequent source of pathogens [17]. *Vibrio parahaemolyticus* is one example and the causative agent of gastroenteritis in humans, but recently also of early mortality syndrome (EMS) in shrimp cultures, causing substantial economic loss [18].

Recruiting quantum physics packed into portable biosensors to unmask pathogens has gotten rather popular recently. Surface-enhanced Raman scattering (SERS) for example has been used to identify Ebola, Lassa, and malaria in a single blood sample in less than half an hour [19]. Nuclear magnetic resonance spectroscopy (NMR), which relies on the unique electromagnetic signature of a nanoparticle coupled either to specific antibodies or primers has been proposed for the detection of various microorganisms [20,21].

Here, Hash et al. compare a combination of NMR and molecular biology with classical real-time PCR for the detection of *V. parahaemolyticus* in shrimp [22]. The authors conclude that the NMR biosensor constitutes a fast, sensitive, and specific

detection tool for EMS-causing V. *parahaemolyticus* that could be used for diagnostic and quality control.

In situ repair of all-ceramic dental restorations by laser irradiation

Nowadays, in dental restoration, all-ceramic crowns and bridges are used instead of metal components, mainly for aesthetic reasons [23]. Moreover, in order to minimise intervention effort, cost and patient discomfort, the trend in mending fractures or chippings of these restorations has recently shifted towards *in situ* repair using laser surface treatment [24].

In the present study, Tokar et al. examine the repair bond strength between composite resin and zirconia/porcelain surfaces subjected to various yttrium scandium gallium garnet (Er,Cr:YSGG) laser pulses [25]. According to the authors, the application of Er,Cr:YSGG laser pulses increases the repair bond strength between zirconia and composite resin with a slightly better result for short pulse rates, despite the control method of grinding with diamond bur still exhibiting the best results.

Conflicts of interest

The author declares no conflict of interests.

REFERENCES

[1] Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics 2016;107:1–8.
[2] Scotti MM, Swanson MS. RNA mis-splicing in disease. Nat Rev Genet 2016;17:19–32.
[3] Benzaquen J, Heeke S, Janho dit Hreich S, Douguet L, Marquette CH, Hofman P, et al. Alternative splicing of P2RX7 pre-messenger RNA in health and diseases: myth or reality? Biomed J 2019;42:141–54.
[4] Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. Nature 2014;509:310–7.
[5] Liu L, Liu S, Song X, Zhu Q, Zhang W. Effect of Nd: YAG laser irradiation on surface properties and bond strength of zirconia ceramics. Lasers Med Sci 2013;30:627–34.
[6] Ferguson SSG, Feldman RD. β-adrenoceptors as molecular targets in the treatment of hypertension. Can J Cardiol 2014;30:S3–8.
[7] Wachter SB, Gilbert EM. Beta-adrenergic receptors, from their discovery and characterization through their manipulation to beneficial clinical application. CRD 2012;122:104–12.
[8] Wang T, Li Y, Lu HL, Meng QW, Cai L, Chen XS. β-Adrenergic receptors : new target in breast cancer. Asian Pac J Cancer Prev 2015;16:8031–9.
[9] Hansson L. The use of propranolol in hypertension: a review. Postgrad Med J 1976;52(Suppl. 4):77–80.
[10] Montoya A, Amaya CN, Belmont A, Diab N, Trevino R, Villanueva G, et al. Use of non-selective β-blockers is associated with decreased tumor proliferative indices in early stage breast cancer. Oncotarget 2017;8:6446–60.
[11] Montoya A, Varela-Ramirez A, Dickerson E, Pasquier E, Torabi A, Aguilera R, et al. The beat adrenergic receptor antagonist propanolol alters mitogenic and apoptotic signaling in late stage breast cancer. Biomed J 2019;42:155–65.
[12] He RH, He YJ, Tang YJ, Zhou HH, McLeod HL, Liu J. The potential anticancer effect of beta-blockers and the genetic variations involved in the interindividual difference. Pharmacogenomics 2016;17:74–9.
[13] Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res 2014;9:18.
[14] Deegan AJ, Hendrikson WJ, El Hai A, Rouwkema J, Yang Y. Regulation of endothelial cell arrangements within hMSCHUVEC co-culture aggregates. Biomed J 2019;42:166–77.
[15] Wu X, Wu W, Pan W, Wu L, Liu K, Zhang H-L. Acute necrotizing encephalopathy: an underrecognized clinicoradiologic disorder. Mediat Inflamm 2015;2015:792578. 10.
[16] Lin YY, Lee KY, Ro LS, Lo YS, Huang CC, Chang KH. Clinical and cytokine profile of adult acute necrotizing eencephalopathy. Biomed J 2019;42:178–86.
[17] Kim HW, Hong YJ, Jo JI, Ha SD, Kim SH, Lee HJ, et al. Raw ready-to-eat seafood safety: microbiological quality of the various seafood species available in fishery, hyper and online markets. Lett Appl Microbiol 2017;64:27–34.
[18] Lai HC, Ng TH, Ando M, Lee CT, Chen IT, Chuang JC, et al. Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. Fish Shellfish Immunol 2015;47:1006–14.
[19] Sebba D, Lastovich AG, Kuroda M, Fallows E, Johnson J, Ahouidi A, et al. A point-of-care diagnostic for differentiating Ebola from endemic febrile diseases. Sci Transl Med 2018;10:eaat0944–1033.
[20] Mlynárik V. Introduction to nuclear magnetic resonance. Anal Biochem 2017;529:4–9.
[21] Luo Y, Alocilja EC. Portable nuclear magnetic resonance biosensor and assay for a highly sensitive and rapid detection of foodborne bacteria in complex matrices. J Biol Eng 2017;11:14.
[22] Hash S, Martinez-Viedma MP, Fung F, Han JE, Yang P, Wong C, et al. Nuclear magnetic resonance biosensor for rapid detection of *Vibrio parahaemolyticus*. Biomed J 2019;42:187–92.
[23] Manziuc M-M, Gasparik C, Burde AV, Colosi HA, Negucioiu M, Dudea D. Effect of glazing on translucency, color, and surface roughness of monolithic zirconia materials. J Esthetic Restor Dent 2019;13:1.
[24] Liu L, Liu S, Song X, Zhu Q, Zhang W. Effect of Nd: YAG laser irradiation on surface properties and bond strength of zirconia ceramics. Lasers Med Sci 2013;30:627–34.
[25] Tokar E, Polat S. Repair bond strength of composite to Er,Cr:YSGG laser irradiated zirconia and porcelain surfaces. Biomed J 2019;42:193–9.